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First record of *Leodice uschakovi* (Wu, Sun & Liu, 2013) (Eunicidae, Polychaete) in Gulf of Mannar, southeast coast of India

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Abstract. We record the polychaete species *Leodice uschakovi* (Wu, Sun & Liu, 2013) for the first time from the two islands of Gulf of Mannar, India. Our find significantly expands the species' geographic range, as it was previously documented only from waters of Hainan Island, southern China, and El Nido, Philippines. A large number of samples was collected, many more than by previous investigations, from dead corals. We provide detailed morphological data, including some previously overlooked key characters, as well as molecular data and intraspecific characters within the species.

Key words. Intraspecific variation, Musal Tivu, new record, polychaete, Pumurichan Tivu

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INTRODUCTION

Among the polychaete families, the Eunicidae comprises 453 species belonging to 11 genera reported worldwide but only 32 species belonging to seven genera reported in Indian waters, namely *Eunice* Cuvier, 1817, *Leodice* Lamarck, 1818, *Lysidice* Lamarck, 1818, *Marphysa* Quatrefages, 1865, *Nicidion* Kinberg,1865, *Palola* Gray in , 1847, and *Paucibranchia* Molina-Acevedo, 2018 (Sivadas and Carvalho 2020). Of these 32 species, at least four are questionably recorded from India: *Eunice floridana* (Pourtalès, 1867), *Lysidice ninetta* Audouin & Milne Edwards, 1833, *Paucibranchia fallax* (Marion & Bobretzky, 1875), *Marphysa sanguinea* (Montagu, 1813), and one has the type locality in Indian waters *M. gravelyi* Southern, 1921. Four species have been documented in India, which have their type localities in Europe: *L. ninetta* from Îles Chausey, France; *M. sanguinea* from Devon, United Kingdom; *Palola siciliensis* from Sicily, Italy; and *P. fallax* from Marseille, France (Wehe and Fiege 2002; Sivadas and Carvalho 2020).

Identifying Indian polychaetes pose a significant challenge due to the influence of the works by Fauvel (1953) and Day (1967). Both staunchly advocated the thought at the time that polychaeta species are mostly cosmopolitan (Hutchings and Kupriyanova 2018). For instance, Fauvel's (1953) book on the polychaetes of India rely heavily on illustrations and species' names from his earlier *Fauna de France* series (Fauvel 1923, 1927). Day's (1967) monograph on South African polychaetes includes numerous European cosmopolitan species, and it is the reference primarily followed by Indian polychaete taxonomists. Thus, there is the possibility that numerous undescribed polychaete species exist in Indian waters, which have been incorrectly attributed to European species. The paucity of up-to-date taxonomic publications on polychaetes of Indian waters exacerbates these challenges, making identification of species difficult.

The genus *Leodice* has a cosmopolitan distribution. It was thought to be a synonym of *Eunice* until 2014, when Zanol et al. (2014) resurrected it as a valid genus on the basis of morphological and genetic data. In Indian waters, only three *Leodice* species have been reported: *Leodice antennata* Savigny in Lamarck, 1818, *L. australis* (Quatrefages, 1866), and *L. laticeps* (Ehlers, 1818), which were considered to belong to *Eunice* until 2014 (Day 1967, 1973; Parulekar 1971; Nageswara and Soota 1981; Srikrishnadhas et al. 1987; Rao and Sastry 2005; Rajasekaran and Fernando 2012; Sivaleela and Venkataraman 2012; Pati et al. 2015; Sivadas and Carvalho 2020).

According to Zanol et al. (2014), species bearing five prostomial appendages, peristomial cirri,

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subacicular hooks, and at least one of the following characteristics, belong to *Leodice*. They are distinguished from the genera *Eunice* and *Nicidion* by the following charactrers: a) regularly articulated prostomial appendages; b) tridentate compound falciger chaetae; c) bi- or tridentate light subacicular hooks. Almost all *Eunice* species assigned to the A and C groups of Fauchald (1970) are expected to be *Leodice* species (Zanol et al. 2014).

Leodice uschakovi (Wu, Sun & Liu, 2013) was described as *Eunice uschakovi* for the first time from Hainan Island, Southern China (Wu et al. 2013), and it was later reportded from the Philippines by Díaz and López (2020). In this study, we report *L. uschakovi* for the first time from Indian waters. We provide a thorough morphological description and illustrations to characterize the species. Additionally, we provide taxonomic intraspecific variation of the key characters based on the samples obtained from the Gulf of Mannar (GOM hereafter) and earlier reports.

METHODS

We collected specimens of *Leodice uschakovi* from November 2022 to September 2023 at depths of 0.5–1.5 m along the shoreline of two islands, Pumurichan Tivu (09°14′14.65″N, 079°11′38.92″E) and Musal Tivu (09°12′01.00″N, 079°04′53.00″E), GOM.

Specimens were collected in crevices of rocks and dead corals. On shore, we washed the rocks and corals to dislodge polychaete specimens. After being anesthetized with isotonic 8% $MgCl_2$ dissolved in freshwater, some specimens for morphological examination were fixed in a 5% formaldehyde–seawater solution and transferred 70% ethanol for preservation. Other specimens for molecular analysis were washed thoroughly in distilled water then fixed in 100% ethanol.

Morphological analysis. Genus-level identification was made by examining the morphological features under a Zeiss Stemi 2000C stereomicroscope. Very tiny characteristics, such as chaetae and subacicular hooks, were studied using a Leica DM 2500 LED compound microscope.

Parapodia were removed using the model proposed by Zanol et al. (2014) from different parts of the body (P1–P6). The specimens were measured using the method proposed by Fauchald (1992), which includes measuring the length from prostomium to the 10th chaetiger and body width at chaetiger 10 including the parapodia. Specimens for examination by scanning electron microscopy were put in a 0.1 mol/L cacodylate buffer, fixed in 1% osmium tetroxide (O_sO_4) for 1 h, rinsed with distilled water, dehydrated in a graded ethanol series (20–100%), and finally transferred to hexamethyldisilazen to complete the dehydration process. The prepared specimens were mounted on aluminum stubs and sputter coated with platinum or palladium using a high-resolution fine coater (JFC-2300HR) before being examined under a Carl Zeiss Sigma-300 field-emission scanning electron microscope at the Centralized Instrumentation Facility of Annamalai University.

The key characters and intraspecific variations of *L. uschakovi* were studied follow Wu et al. (2013), Zanol et al. (2014, 2020), and Díaz and López (2020). The description provided here is based on the morphology of all specimens collected in our study. Altogether, we collected 12 specimens from the two islands. Two specimens have been deposited in the Museum of the National Bureau of Fish Genetic Resources (Indian Council of Agricultural Research; **NBFGR-ICAR**), Lucknow, India. The remaining 10 specimens have been deposited at the reference Museum of Centre of Advanced Study in Marine Biology (**CASMB-AU-RM**). We also undertook a detailed literature survey of the genus *Leodice* (Faulchad 1992; Carrera-Parra and Salazar-Vallejo 1998; Zanol et al. 2007, 2014, 2020; Choi et al. 2017; Bergamo et al. 2018).

To investigate the intraspecific morphological variation, we studied nine specimens. We compare our specimens with those reported from Hainan Island (three specimens observed) (Wu et al. 2013) and the Philippines (one specimen observed) (Díaz and López 2020).

Molecular analysis. We extracted genomic DNA from the anterolateral segment of two specimens using the NucleoSpin Tissue Kit (Macherey-Nagel) following the manufacturer's instructions. The targeted region for amplification was approximately 600 base pairs of the cytochrome c oxidase subunit I (COX1) gene, employing the poly LCO and poly HCO COX1 primers. A polymerase chain reaction (PCR) analysis was carried out in a total volume of 10.5 µL, comprising 5 µL of 2X Phire Master Mix, 0.25 µL of each primer (final concentration 0.2µM), 1 µL of DNA, and 4 µL of distilled water (Folmer et al. 1994)

The PCR amplification occurred in a GeneAmp PCR System 9700 (Applied Biosystems) thermal cycler, following the temperature profile: 98 °C for 30s; 98 °C for 5 s, 45 °C for 10 s, and 72 °C for 15 s for 10 cycles; 98 °C for 5 s, 50 °C for 10 s, and 72 °C for 15 s for 30 cycles; 72 °C for 60 s; and 4 °C. Subsequently, PCR products underwent purification using ExoSAP-IT; specifically, 5 µL of PCR product was mixed with 0.5 µL of ExoSAP-IT, incubated at 37 °C for 15 min, followed by enzyme inactivation at 85 °C for 5 min (ExoSAP-IT, User Guide, GE Healthcare).

Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing Kit v. 3.1 (GeneAmp PCR System 9700, Applied Biosystems). We deposited our two new sequences in GenBank. Multiple alignments of these sequences with those of other *Leodice* species were conducted using the Clustal W method within BioEdit v. 7.2.6 (Hall 1999). Pairwise genetic distances were calculated using

Kimura-2-parameter model in MEGAX- (64 bit) (Kumar et al. 2018). The best-fit model for the data is GTI + I +G, as designated by the Akaike criterion in JModelTest v. 2 implemented in CIPRES Science Gateway (Miller et al. 2010). Bayesian analyses were run in MrBayes v. 3.2.6 (Ronquist and Huelsenbeck 2003) implemented in the CIPRES Science Gateway from 2×10^6 generations; trees sampled every 100th generation and 25% discarded as burn-in. Convergence of the runs was verified by average standard deviation <0.01.

RESULTS

Order Eunicida
Family Eunicidae Berthold, 1827
Genus *Leodice* Lamarck, 1818

***Leodice uschakovi* (Wu, Sun & Liu, 2013)**
Figures 1–3

Type locality. Southern China Sea, Hainan Island.

Materials examined. INDIA – TAMILNADU • Ramanathapuram, Gulf of Mannar, Pumurichan Tivu Island; 09°14′14.65″N, 079°11′38.92″E; 0.5–1.5 m depth; 23.XI.2022; Sasmita Swain leg.; on dead coral; 2 spec. (1 in formaldehyde, EUNLUSC.1/NBFGR; 1 in ethanol, EUNLUSC.2/NBFGR) • same locality; 5.IV.2023; Sasmita Swain leg.; dead coral; 8 spec. (1 in ethanol, CASMB-AU-RM-BEN101; 1 in ethanol, CASMB-AU-RM- BEN102; 1 in ethanol, CASMB-AU-RM-BEN103; 1 in ethanol, CASMB-AU-BEN104, GenBank PP280551; 1 in ethanol, CASMB-AU-BEN105; 3 in ethanol, CASMB- AU-BEN106) – TAMILNADU • Gulf of Mannar, Musal Tivu Island; 09°12′01.00″, 079°04′53.00″E; 1.0–1.5 m depth; 17.IX.2023; Sasmita Swain leg.; on dead coral; 2 specimens (1 in ethanol, CASMB-AU-BEN107; 1 in ethanol, CASMB-AU-BEN108, GenBank PP280552).

Description. Complete specimens: EUNLUSC.1/NBFGR, 130 chaetigers; total length 7.4 cm, length from prostomium to 10th chaetiger 1.5 cm, width at chaetiger 10 including the parapodia 0.4 cm; EUNLUSC.2/NBFGR, 114 chaetigers; total length 6.9 cm, length through 10th chaetiger 1.4 cm, width 0.4 cm at chaetiger 10, including parapodia: CASMB-AU-BEN107; 155 chaetigers; total length 8.1 cm, length through 10th chaetiger 1.2 cm, width 0.4 cm at chaetiger 10, including parapodia.

Incomplete, missing pygidium and some posteriors chaetigers: CASMB-AU-RM-BEN101; 98 chaetigers; length through 10th chaetiger 1.3 cm, width 0.4 cm at chaetiger 10, including parapodia: CASMB-AU-RM-BEN102; 102 chaetigers; length through 10th chaetiger 1.1cm, width 0.4 cm at chaetiger 10, including parapodia: CASMB-AU-RM-BEN103; 97 chaetigers; length through 10th chaetiger 1.4 cm, width 0.4 cm at chaetiger 10, including parapodia: CASMB-AU-BEN104; 98 chaetigers; length through 10th chaetiger 1.5 cm, width 0.5 cm at chaetiger 10, including parapodia: CASMB-AU-BEN105; 68 chaetigers; length through 10th chaetiger 1.4 cm, width 0.4 cm at chaetiger 10, including parapodia: CASMB-AU-BEN106(3); 93 chaetigers, 98 chaetigers and 84 chaetigers; length through 10th chaetiger 1.4 cm and 1.3 cm, width 0.4 cm at chaetiger 10, including parapodia: CASMB-AU-BEN108; 105 chaetigers; length through 10th chaetiger 1 cm, width 0.5 cm at chaetiger 10, including parapodia.

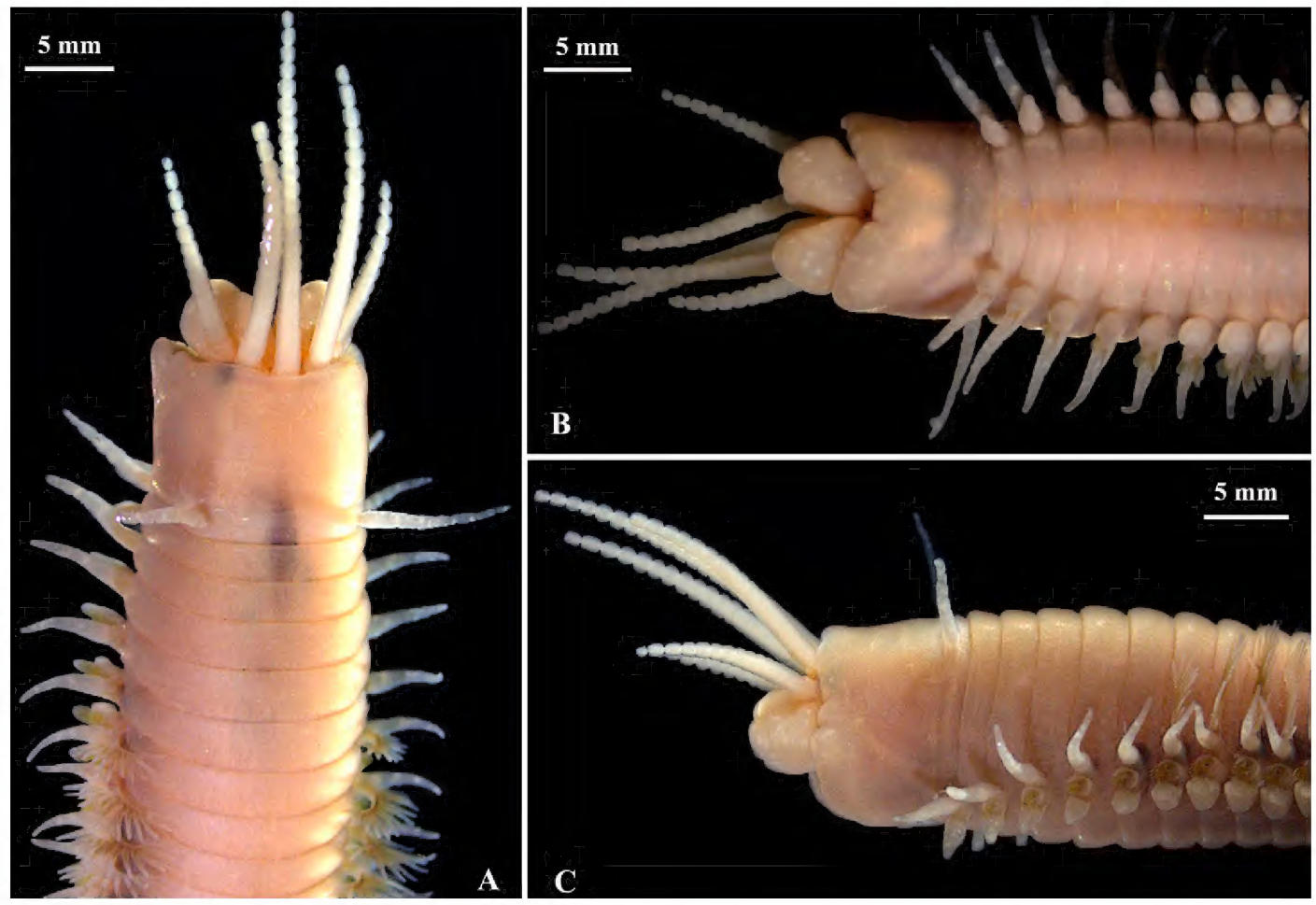

Figure 1. *Leodice uschakovi*, CASMB-AU-BEN105. **A.** Anterior end, dorsal view. **B.** Anterior end, ventral view. **C.** Anterior end, lateral view.

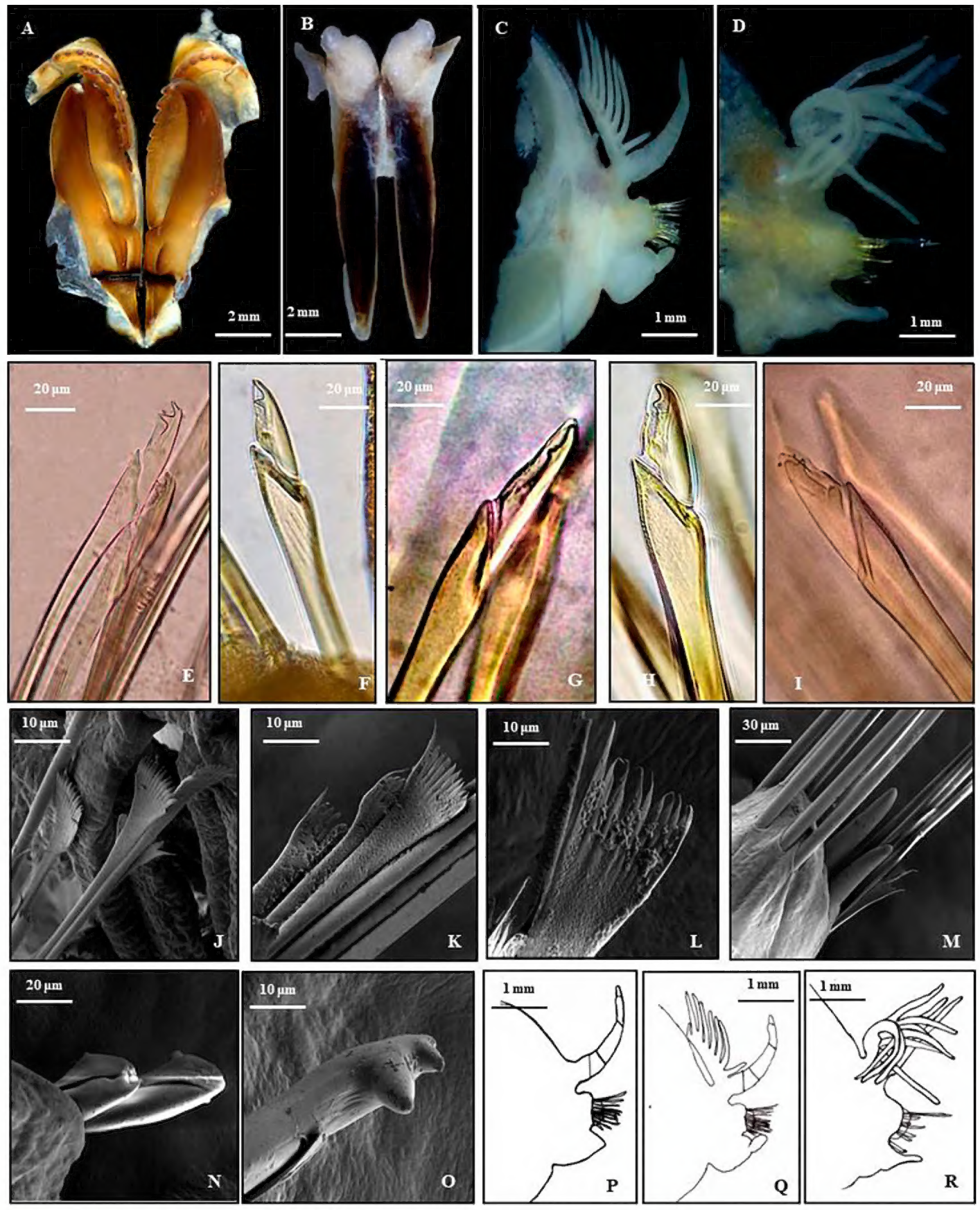

Figure 2. *Leodice uschakovi* CASMB-AU-BEN105, EUNLUSC.1/NBFGR. **A.** Maxillae, dorsal view. **B.** Mandible, ventral view. **C.** Chaetiger 18, anterior view. **D.** Chaetiger 90, anterior view. **E.** Tridentate falciger, chaetiger 62. **F.** Type I bidentate falciger, chaetiger 26. **G.** Type II bidentate falciger, chaetiger 26. **H, I.** Type III bidentate falciger, chaetiger 30. **J.** Pectinate chaetae with 13 teeth, chaetiger 37. **K.** Pectinate chaetae with 11 teeth, chaetiger 32. **L.** Pectinate chaetae with 10 teeth, chaetiger 82. **M.** Aciculae, chaetiger 32. **N.** Pair of subacicular hook, chaetiger 82. **O.** Subacicular hook, chaetiger 32. **P.** Chaetiger 3, anterior view. **Q.** Chaetiger 18, anterior view. **R.** Chaetiger 90, anterior view.

Prostomium distinctly narrower and nearly half as deep as peristomium with deep median sulcus (Figure 1A, B); with anterior margin truncate and inflated dorsally. Five regularly articulated prostomial appendages, arranged in semicircle shape (Figure 1A); both median and lateral antennae in line, palps anterior and slightly lateral to lateral antennae; palps, median and lateral antennae evenly spaced from one another. Ceratophores and palpophores short and ring shaped, without articulations; length of prostomial appendages gradually increases from palps to median antennae. Median antennae with 17–23 articulations moniliform reaching middle of 5th chaetiger or anterior end of 6th chaetiger; lateral antennae have 10–17 moniliform articulations reaching to posterior end of first chaetiger or one third of the second chaetiger; palps with 7–9 moniliform articulations reaching till anterior edge posterior peristomial ring. Eyes posterior to palps, covered by anterior end of the peristomium (Figure 1A). Peristomium cylindrical with distinct inflated lateroventral lips (Figure 1A, B); peristomial ring visible on all sides; anterior peristomial ring three times larger than peristomium ring. Peristomial cirri slender, tapering, with 7–8 articulations reaching end third chaetiger posteriorly and extending all the way to anterior end peristomium.

Maxillary formula 1+1, 5+6–7, 6–7+0, 5–6+9, 1+1 (Figure 2A); MxVI absent, Mx III part of distal arc with left MxIV. Maxillary carrier approximately 2.5 times smaller than MxI; anterior region rectangular and parallel to each other, posterior end it gradually tapering with a pair of lobular wings at lateral of maxillary carrier; MxI fang like, falcal arch not extended, basal inner edge with curvature where MxII posterior most end fits; MxII wide, with triangular recurved teeth, seems half of its length toothed; MxIII short, curved, part of distal arc, situated ventral to left Mx II; left MxIV with wide rounded base, 2nd and 3rd basal teeth from left to right longer than other teeth; MxV rectangular, a little wider than its long, with a short blunt tooth on top of maxilla. Mandibles dark, cutting plates whitish in color, without distinct growth rings (Figure 2B).

Branchiae starts from 5th or 6th chaetiger (Figure 1A, C) to end body, ends at 3rd to 4th chaetiger before

pygidium. First branchia with three filaments equivalent to one-third of notopodial cirri length; all branchiae erect and pectinate; 2nd branchia has nine filaments; maximum 10 filaments with thick, tapering branchial stem, around 1.5 times longer than longest filament present at 19th and 21st chaetiger, and as long as notopodial cirri. Except first branchiae, each branchiae has 6–10 filaments; filament number reduces suddenly, last few branchiae with only four visible filaments present around one-third of notopodial cirri length. No single filament throughout body.

Chaetal lobe asymmetrically rounded. Anterior chaetal lobe nearly symmetrical, with gradually asymmetrically rounded at middle and posterior chaetiger with elongated ventral portion. Prechaetal lobes of anterior and middle chaetigers have obliquely transverse folds, while posterior chaetigers are simply following the shape of the chaetal lobe. Postchaetal lobes anterior and posterior chaetigers follow the shape of the chaetal lobe, while the median chaetigers reach beyond the chaetal lobe, which is visible in anterior view.

Notopodial cirri with three or four articulations throughout the body. Ventral cirri anterior chaetigers protruded conically with inflated ovate bases starting from the third chaetiger (Figure 2P), while the middle chaetigers (starting in 35–37 chaetiger) are thick, digitiform, with inflated ovate bases reduced a little bit, and ventral cirri more prominent (Figure 2C, Q); ventral cirri of posterior chaetigers slender and digitiform, their inflated bases disappear (Figure 2D, R).

Limbate chaetae slender tapering and present in all parapodia. Pectinate chaetae with thin shaft, flat with 10–13 teeth (Figure 2J–L), one marginal tooth longer than the other teeth. Shafts of all compound falcigers are inflated and marginally serrated; anterior and middle compound falcigers relatively thick, tapering. Three types of bidentate falcigers; type I: proximal tooth triangular, slender, directed laterally; distal tooth triangular, longer and thicker than proximal tooth and directed distally (Figure 2F), it appears all over body, subacicular region near ventral cirri; type II: proximal tooth tapering very small look little inflated from base, directed laterally, distal tooth becoming narrow, tapering, longer than proximal tooth and directed distally (Figure 2G), mostly appear in anterior and middle chaetigers; type III: proximal tooth tapering, smaller than the distal tooth, directed laterally; distal tooth tapering, longer and directed distally (Figure 2H, I), appears all over the body; in posterior chaetigers bidentate falcigers type III number decreased. Anterior and middle compound falciger starting in 32–75 chaetiger tridentate with small third tooth closely appear to second tooth; proximal tooth triangular, slender, directed laterally with slightly basally; second tooth triangular, slender, directed obliquely distally; guards rounded, bluntly pointed, and marginally serrated (Figure 2E). Aciculae tapering, relatively blunt or bifid tips; where bifid aciculae found mostly at posterior chaetigers; paired in most chaetigers (Figure 2M). Subacicular hooks present from chaetiger 23–25, with one or two per parapodium (Figure 2N); yellow and tridentate, with third tooth in a crest, shaft straight; proximal tooth triangular, distally blunt, directed laterally, larger than distal tooth; guards covering both proximal and distal tooth (Figure 2O).

Pygidium with one pair of anal cirri reaching to last fourth chaetiger with five articulations to its entire length.

Intraspecific variation. The characters studied include i) the start of tridentate compound falciger, ii) the number of filaments in the first branchiae, and iii) the number of teeth in pectinate chaetae. The colour of our preserved specimens vary from grey to pale cream. Invariably, start of tridentate compound falciger varies from chaetiger 32 to 75. In specimens EUNLUSC.1/NBFGR, EUNLUSC.2/NBFGR, and CASMB-AU-BEN107 the tridentate compound falciger starts at chaetiger 69, 36, and 66, respectively; in specimens CASMB-AU-RM-BEN101, CASMB-AU-RM-BEN102, CASMB-AU-RM-BEN103, CASMB-AU-RM-BEN104, CASMB-AU-RM-BEN105 and CASMB-AU-RM-BEN108, the tridentate compound falciger starts at chaetiger 62, 75, 32, 42, 62, and 69, respectively. As for specimens reported from Hainan Island by Wu et al. (2013), the tridentate falciger starts at chaetiger 20; the specimen reported from the Philippines by Díaz and López (2020) had the tridentate falciger starting at chaetiger 43. Like the tridentate compound falciger, the number of filaments present at first branchiae varies from two to seven in our specimens. In CASMB-AU-RM-BEN101, the right side has two filaments, the left side has five filaments, and both are present at the chaetiger 6; in CASMB-AU-RM-BEN108, the right side has three filaments, the left side has seven filaments, and both are present at chaetiger 6. In CASMB-AU-RM-BEN105, there were three and five filaments right and left sides originating at chaetigers 5 and 6, respectively. In Hainan Island specimens, the number of filaments present at first branchiae varies from 2–4 filaments (Wu et al. 2013); data were missing for the specimen studied by Díaz and López (2020). The number of teeth on the pectinate chaetae varies from 10 to 14 in all our specimens, but in Hainan Island specimens had 10 or 11 teeth on the pectinate chaetae. In the specimen from the Philippines the number of teeth was eight or nine. We compare these three characters among our specimens and those reported in the literature in Table 1.

Molecular identity. Both sequenced specimens are sister taxa and have a pairwise genetic distance of zero. Genetically, they are clearly different from the other *Leodice* species that have been sequenced. The minimum distances observed between *L. uschachovi* and *L. antarctica* is 19.85%, and between *L. uschachovi* and *L. harassii*, 20.11% (Table 2). The latter is the sister species to *L. uschachovi* in the current phylogenetic hypothesis (Figure 3).

Habitat. Intertidal (Wu et al. 2013) and the sublittoral at 18 m (Díaz and López 2020), on coral and rocky bottoms.

Table 1. Key Intraspecific variation within *Leodice uschakovi*. Abbreviations: GOM= Gulf of Mannar.

Specimens	Locality	Specimen status, no. of chaetigers	Fixation	Length to chaetiger 10	Prostomium-median sulcus	1st branchial filament	Branchiae starting chaetiger	Branchiae: max. & min. no. of filaments	Start of tridentate falciger	Pectinate chaetae no. of teeth
Leodice uschakovi (Wu et al., 2013)	Hainan Island, China	Complete	70% ethanol	?	Median sulcus deep, V-shaped	2–4 filaments	4 or 5	Maximum 11–15 filaments at chaetiger 7–9	20	10–11
Leodice uschakovi (Díaz and López; 2020)	El Nido Island, Philippines	Incomplete and complete	70% ethanol	0.49 cm in incomplete specimen	Median sulcus discrete	?	6	?	43	8–9
EUNLUSC.1/NBFGR	Pumurichan Island, GOM	Complete	5% formaldehyde	1.5 cm	Median sulcus deep, V-shaped	3 filaments	6	10 maximum at chaetiger 11–13 and 3 minimum at chaetiger 6	69	10–11
CASMB-AU-RM-BEN101	Pumurichan Island, GOM	Incomplete, pygidium and some chaetiger missing	5% formaldehyde	1.3 cm	Median sulcus deep, V-shaped	Right side 2 and left side 5 filaments	6	7 maximum at chaetiger 10 and 2 minimum at chaetiger 6	62	11–13
CASMB-AU-RM-BEN102	Pumurichan Island, GOM	Incomplete, pygidium and some chaetiger missing	5% formaldehyde	1.1 cm	Median sulcus deep, V-shaped	5 filaments	6	10 maximum at chaetiger 15–18 and 4 minimum at chaetiger 99–102	75	10–11
CASMB-AU-RM-BEN103	Pumurichan Island, GOM	Incomplete, pygidium and some chaetiger missing	5% formaldehyde	1.4 cm	Median sulcus deep, V-shaped	5 filaments	6 left side	9 maximum at chaetiger12 and 4 minimum at chaetiger 97	32	10–11
EUNLUSC.2/NBFGR	Pumurichan Island, GOM	Complete	70% ethanol	1.4 cm	Median sulcus deep, V-shaped	7 filaments	6	10 maximum at chaetiger 9–11 and 4 minimum at chaetiger 100–102	36	10–14
CASMB-AU-BEN104	Pumurichan Island, GOM	Incomplete, pygidium and some chaetiger missing	70% ethanol Molecular	1.5 cm	Median sulcus deep, V-shaped	4 filaments	6	9 maximum at chaetiger 10, 11 and 4 minimum at chaetiger 6 and 98	42	10–11
CASMB-AU-BEN105	Pumurichan Island, GOM	Incomplete, most chaetiger are missing	5% formaldehyde	1.4 cm	Median sulcus deep, V-shaped	Right side 3 at 5th chaetiger and left side5 filament at 6th chaetiger	5 or 6	9 maximum at chaetiger11 and 3 minimum at chaetiger 5	62	11–13
CASMB-AU-BEN107	Musal Tivu, GOM	Complete	70% ethanol	1.2 cm	Median sulcus deep, V-shaped	5 filaments	6	7 maximum at chaetiger 10 and 3 minimum at chaetiger 152	66	10–11
CASMB-AU-BEN108	Musal Tivu, GOM	Incomplete, pygidium and some chaetiger missing	70% ethanol Molecular	1 cm	Median sulcus deep, V-shaped	Right side 3 and left side 7 filament	6	10 maximum at chaetiger 12 and 3 minimum at chaetiger 6	69	10–11

Table 2. Key genetic distance (K2P) based COI sequence among 14 *Leodice* species with two outgroup taxa (*Paola viridis* and *Marphysa sanguinea*).

No.	Species with accession number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Data-source
1	PP280551 *Leodice uschakovi*																		Present study
2	PP280552 *Leodice uschakovi*	0.0000																	"
3	MF669544.1 *Leodice duplexa*	0.2643	0.2643																Choi et al. 2017
4	GQ497561.1 *Leodice americana*	0.2331	0.2331	0.2634															Zanol et al. 2010
5	GQ497532.1 *Leodice antarctica*	0.1985	0.1985	0.2369	0.1826														"
6	DQ317858.1 *Leodice antennata*	0.2502	0.2502	0.1804	0.2516	0.2620													Schulze 2006
7	GQ497533.1 *Leodice cf. antillensis*	0.2787	0.2787	0.2710	0.2584	0.2640	0.2881												Zanol et al. 2010
8	GQ497535.1 *Leodice harassii*	0.2011	0.2011	0.2439	0.2198	0.2100	0.2390	0.2142											"
9	GQ497531.1 *Leodice cf. limosa*	0.2847	0.2847	0.2918	0.2540	0.2883	0.2831	0.2937	0.2500										"
10	GQ497529.1 *Leodice lucei*	0.2625	0.2625	0.1982	0.2601	0.2492	0.2028	0.2743	0.2198	0.2569									"
11	GQ497530.1 *Leodice miurai*	0.2609	0.2609	0.2471	0.2828	0.2666	0.2518	0.2887	0.2328	0.3005	0.2125								"
12	GQ497528.1 *Leodice rubra*	0.2418	0.2418	0.1780	0.2265	0.2453	0.0662	0.2693	0.2304	0.2688	0.2057	0.2555							"
13	GQ497563.1 *Leodice thomasiana*	0.2488	0.2488	0.2664	0.2383	0.2520	0.2842	0.2302	0.2007	0.2551	0.2570	0.2838	0.2695						"
14	GQ497539.1 *Leodice torquata*	0.2462	0.2462	0.2664	0.2337	0.2272	0.2441	0.2092	0.2216	0.2628	0.2630	0.2744	0.2303	0.1591					"
15	GQ497534.1 *Leodice valens*	0.2584	0.2584	0.2734	0.2371	0.2341	0.2620	0.2479	0.2254	0.2577	0.2477	0.2418	0.2618	0.2830	0.2494				"
16	GQ497556.1 *Palola viridis*	0.2418	0.2418	0.3048	0.2656	0.2413	0.2689	0.2360	0.2352	0.2559	0.3082	0.2890	0.2544	0.2223	0.1983	0.2741			"
17	GQ497547.1 *Marphysa sanguinea*	0.2825	0.2825	0.2955	0.2937	0.2898	0.2719	0.2991	0.2597	0.3318	0.2717	0.3105	0.2870	0.2828	0.2776	0.2712	0.2807		"

Distribution. Indian Ocean and South China Sea. *Leodice uschakovi* was first discovered from Hainan Island, Southern China Sea (Wu et al. 2013) and later it was reported from El Nido Island in the western

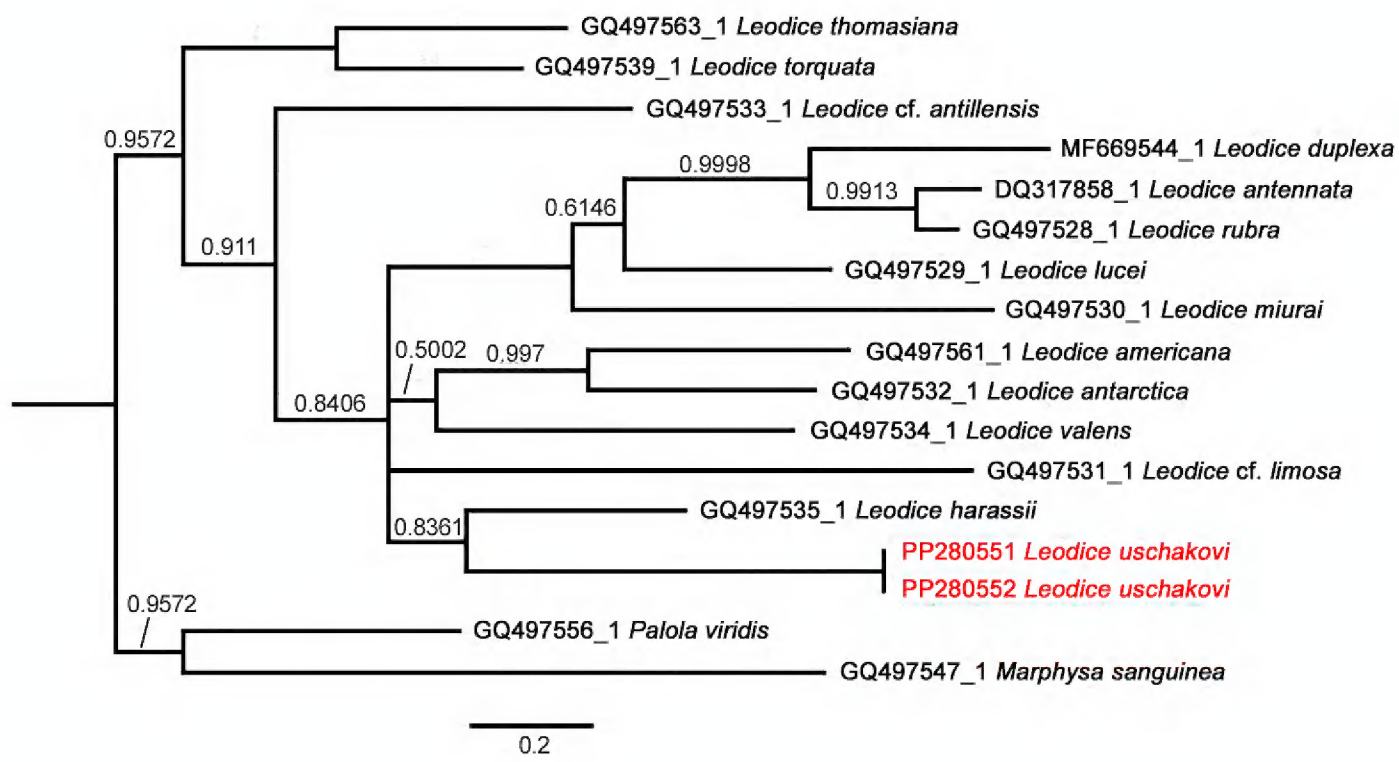

Figure 3. Bayesian-inference tree. Posterior probabilities placed on branches.

Philippines Island (Díaz and López 2020). Here, we record this species from the Indian Ocean for the first time, at Pumurichan Tivu and Musal Tivu islands, in the Gulf of Mannar, India (Figure 4).

DISCUSSION

We found 12 specimens at our collection sites in Indian waters. Our specimens were determined to be *L. uschakovi*, resembling specimens of Wu et al. (2013) and Díaz and López (2020). In addition to the key characters provided by these previous authors, we found additional key characters, and we also noted intraspecific variation (Table 1). These features might have gone unnoticed or overlooked in their investigations due to a lack of specimens, since only three specimens from Hainan Island and one in the Philippines were previously available. In our material, we observed that the compound bidentate falcigers were of three different types (I–III), which are present on most chaetigers.

Knowledge of interspecific variation is crucial for accurate identification, since variation within species frequently leads to ambiguity in identifications. Our study of 12 specimens reveals well all intraspecific variation seen in *L. uschakovi*, including the start of the tridentate compound falcigers, number of filaments in the first branchiae, and number of teeth in pectinate chaetae.

Literature review revealed that only three species of *Leodice*, namely *L. antennata* in the Andaman Sea, Maharashtra, and Gujarat (Parulekar 1971; Nageswara and Soota 1981; Rajasekaran and Fernando 2012), *L. australis* (Quatrefages, 1866) in Tamil Nadu Parangipettai, Gujarat, and the Andaman Sea (Nageswara and Soota 1981; Srikrishnadhas et al. 1987; Rao and Sastry 2005; Rajasekaran and Fernando 2012; Sivaleela and Venkataraman 2012), and *L. laticeps* (Ehlers, 1818) in Maharashtra (Pati et al. 2015) have been recorded in Indian waters (Sivadas and Carvalho 2020). Of these three, *L. antennata* was previously thought to belong to *Eunice*. Since the publications of Zanol et al. (2014, 2020), the remaining species have been placed in *Leodice*.

In the genetic comparison analysis, partial COX1 sequences of 560 bp were acquired from two of our specimens. A broader dataset was assembled, comprising 16 species of eunicids. Among these, 14 were

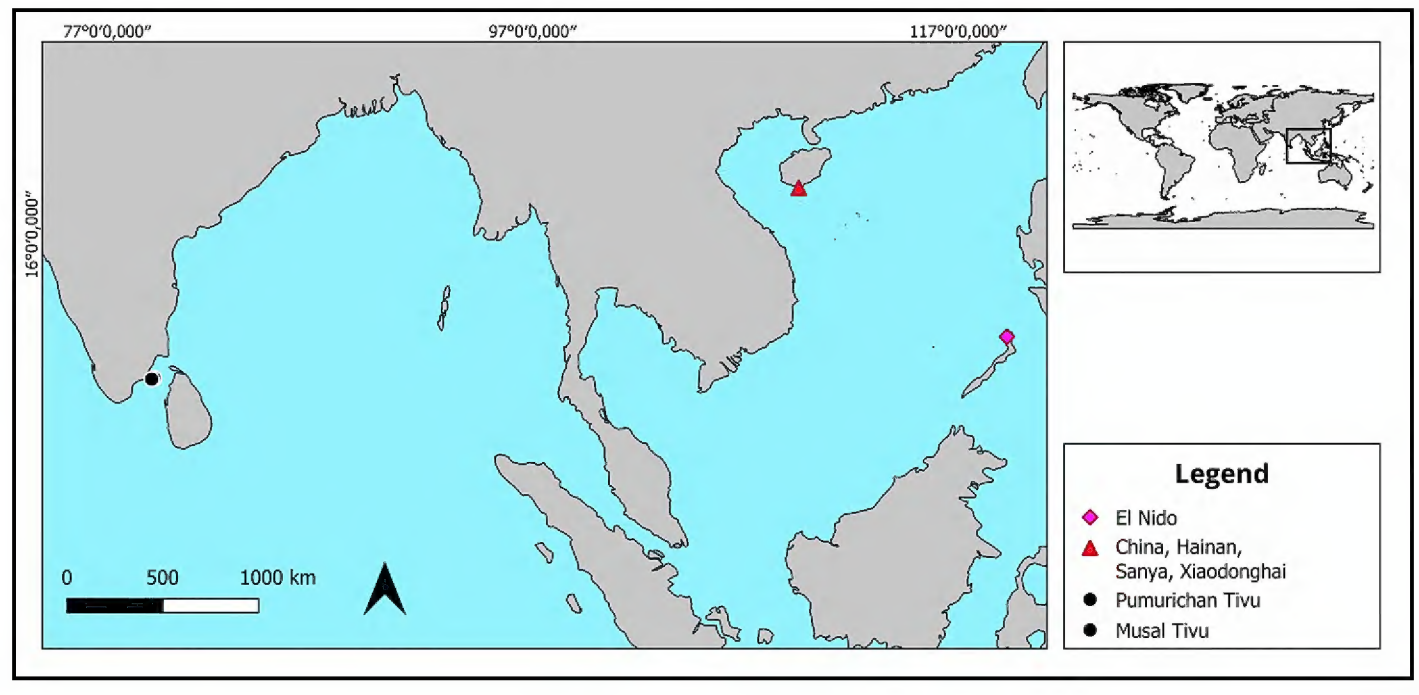

Figure 4. Distribution of *Leodice uschakovi*. Pink diamond = El Nido Island, Philippines; red triangle= Hainan Island, China, black dot = Pumurichan Tivu and Musal Tivu islands, Gulf of Mannar, India.

Leodice species with two out-group taxa, *Palola viridis* and *Marphysa sanguinea*. In *Leodice* species, with 10 designated as *Eunice* species and three as *Leodice* species based on records from GenBank (Schulze 2006; Zanol et al. 2010; Choi et al. 2017). Our Bayesian-inference tree using these genetic data (Figure 3) reveals the placement of *L. uschakovi* among other *Leodice* species. Notably, *L. uschakovi* belongs to a clade including *L. harasii* (Figure 3).

Our additional description of, and findings of intraspecific variation in, *L. uschakovi* has provides a better understanding of the morphology this species. Our new biogeographical data also greatly expands this species geographic range and adds new information on polychaetes of India.

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ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

No ethical statement is reported.

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Author contributions

Conceptualization: SS, PM, TKSS, JZ. Methodology: SS, PM. Investigation and validation: SS, TKSS, JZ Funding acquisition: PM. Visualization: SS, JZ, TKSS, PM. Software: SS, JZ. Writing – original draft: SS, PM. Writing – review and editing: SS, JZ, TKSS, PM.

Data availability

All data that support the findings of this study are available in the main text.

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